

Figure 1

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Hordeum vulgare
Oryza sativa
Hordeum spontaneum
Eleusine coracana
Secale cereale
Triticum durum
Zea mays
Triticum aestivum

10	20	30	40	50
---MASD-HR RFVLSGAVILL	SVLAVAAGATL	E-----	SVKDECQLGV	
---MASN-KV VFSVLLLAIVV	SVLAATATMA	EYHHQDQVYY	TPGPLCQPGM	
---MAFK--Y QLLLSAAVML	AILAATVVT	-----	SFGDMCAPGD	
-----	-----	-----	SVGTSCIPGM	
-----	-----	-----	SVGGQCVPGL	
---MACKSSC SLLLLAAVIL	SVLAA--A--	-----	SASGSCVPGV	
MASSSSSSSHR RLILAAAVIL	SVLAASA--	-----	SAGTSCVPGW	
---MASN-HR RFLLSGAVILL	SVLAAVAA-L	E-----	SVEDECQPGV	

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	60	70	80	90	100
DFPHNPLATC	HTYVIKRVCG	--RGPSRPM	LV-----	-----	KERC
GYPMYPLPRC	RALVKRQCVG	--RGTAAAA	EQ-----	-----	VRRDC
ALPANPLRAC	RTYVVSQIC	--HVGPRLST	WD-----	-----	MKRRC
AIPHNPILDSC	RWYVAKRACG	--VGPRLAT	QE-----	-----	MKARC
AMPHNPLGAC	RTYVVSQIC	--HVGPRLFT	WD-----	-----	MKRRC
AFRTNLLPHC	RDYVLQQTCG	TFTPGSKLPE	WMTSASIYSP	GKPYLAKLYC	
AIPHNPPLPSC	RWYVTSRTCG	--IGPRLPW	PE-----	-----	LKRRC
AFPHNALATC	HTYVIKRVCG	--RGPSRPM	LV-----	-----	KERC

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	110	120	130	140	150
CRELAAVP-D	HCRCEALRL	MDGVRTPE--	---GRVVEG	RLGDERRDCPR	
CRQLAAVDDS	WCRCEAISHM	LGGIYRELG-	---APDVGH	MSEVFRGCR	
CDELSAIP-A	YCRCEALRII	MDGTVTWQ--	---GVFG-A	YFKDMPNCPR	
CROLEAIP-A	YCRCEAVRIL	MDGVVTP--	---SGQHEGR	LLQDLPGCPR	
CDELLAIP-A	YCRCEALRL	MDGVVTQQ--	---GVFEGG	YLKDMPNCPR	
CQELAEIS-Q	QCRCEALRYF	IALPVPSQPV	DPRSGNVGES	GLIDLPGCPR	
CRELADIP-A	YCRCTALSIL	MDGAIPPGP-	---DAQLEGR	-LEDLPGCPR	
CRELAVVP-D	YCRCEALRVL	MDGVRAEE--	---GHVVEG	RLGDERRDCPR	

Hordeum vulgare
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Hordeum spontaneum
Eleusine coracana
Secale cereale
Triticum durum
Zea mays
Triticum aestivum

	160	170	180	190
EEQRAFAATL	VTAACNLSS	VQAPGVRLVL	LADG-----	
GDLERAAASL	PAFCNVDIPI	GGG-GVCYWL	ARSGY-----	
VMQTSYAANL	VNPQECNLWT	IHGSPSCP	QPGYEVVL--	
QVQRAFAPKL	VTEVECNLAT	IHGGPFCLSL	LGAGE-----	
VTQRSYAATL	VAPQECNLPT	IHGSPYCPTL	QAGY-----	
EMQWDFVRL	VAPGQCNLAT	IHNVRYCPAV	EQPLWI----	
EVQRGFAATL	VTEAECNLAT	ISGVAECPWI	LGGGTMPSK-	
EAQREFAATL	VTAACNLPT	VS--GVGSTL	GATGRWMTIE	LPK

Figure 2

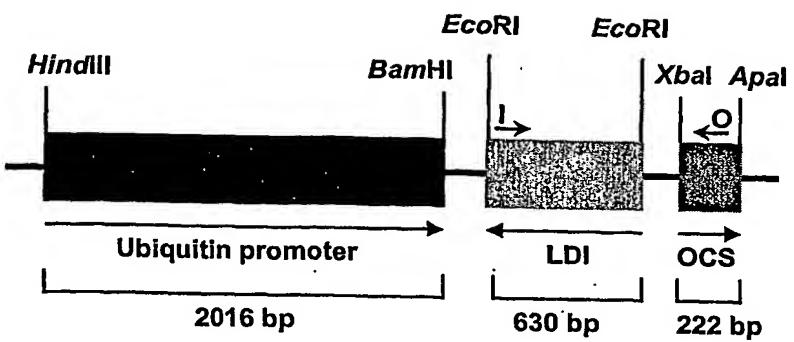


Figure 3

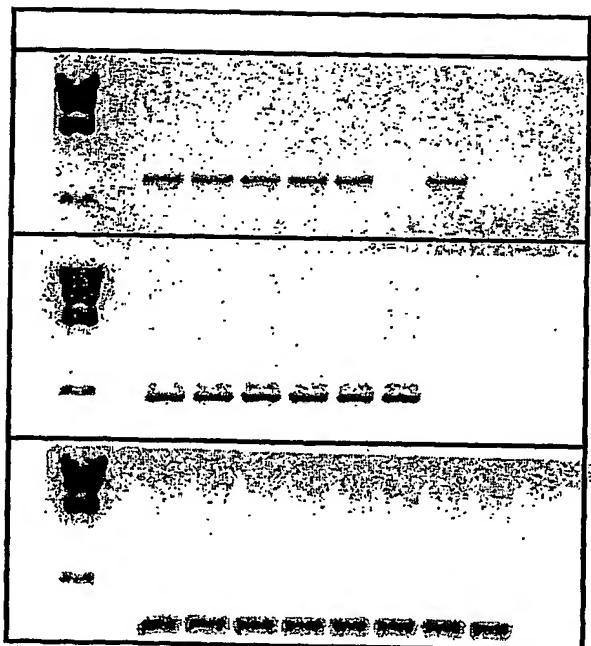
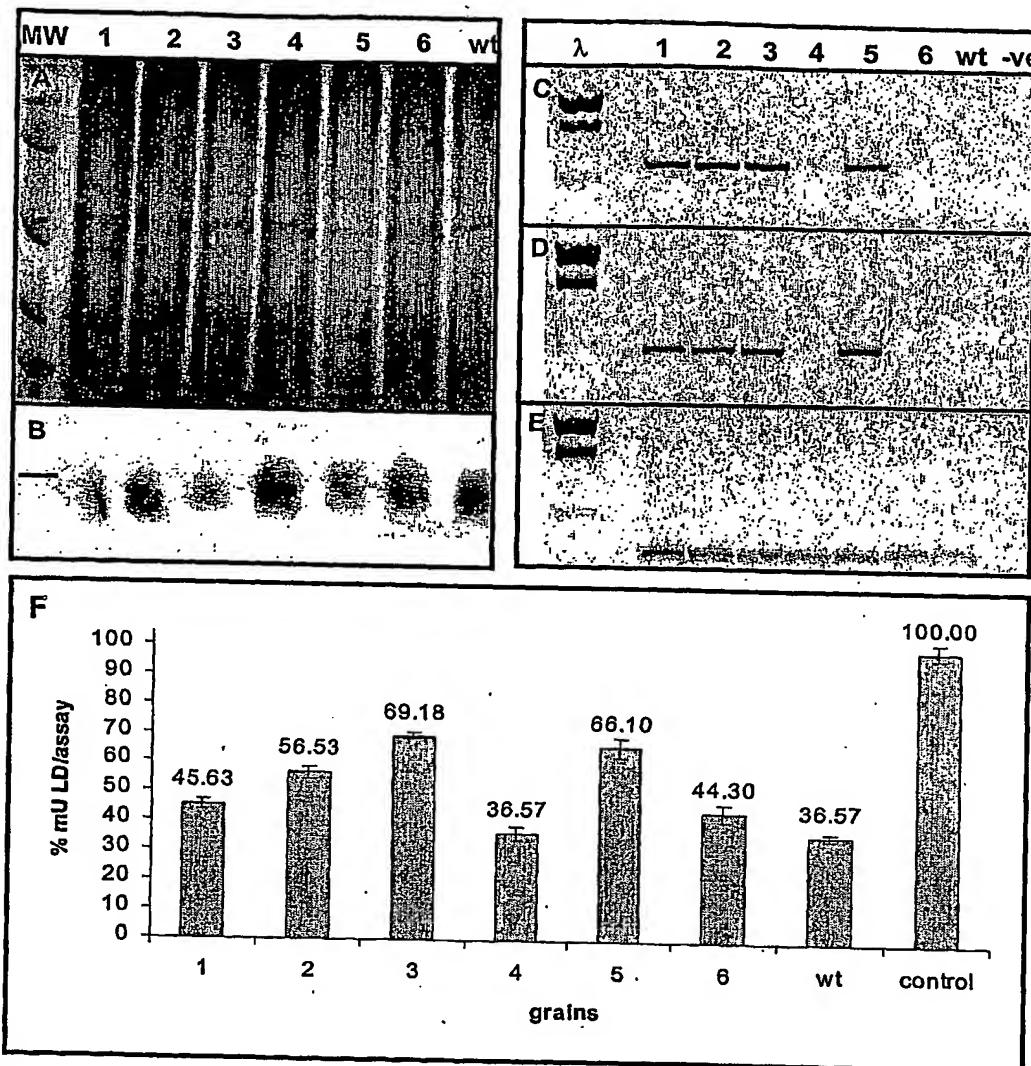


Figure 4



(A) 10% SDS-PAGE of LDI extracts corresponding to 5 µg protein.

(B) Immunoblot developed with antisera against LDI; bar represents 21.5 kD.

(C) PCR of genomic DNA with primers Inhib-6 + OCS-II for the *LDI* gene in antisense direction (817 bp).

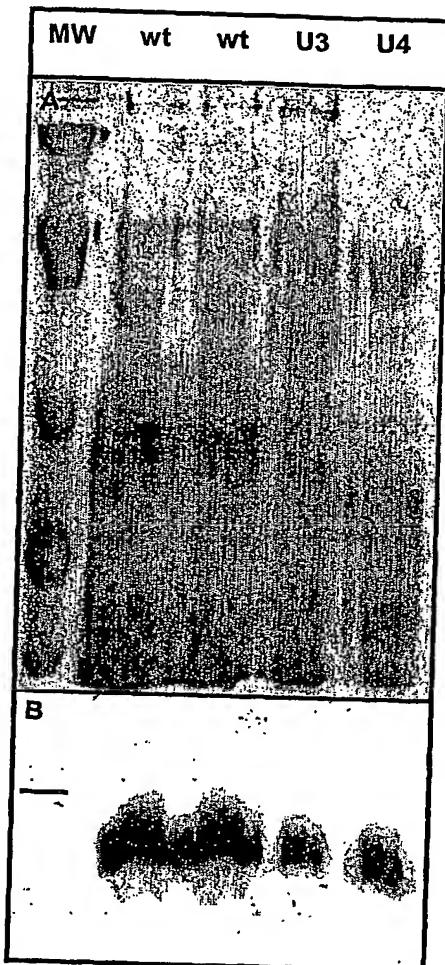
(D) PCR of genomic DNA primers BAR-I + BAR-II for the *bar* gene (534 bp).

(E) PCR of genomic DNA primers TUB-F + TUB-R for the *tubulin* gene (217 bp).

(F) LDI activity assay. The control represents the amount of LD used for each assay. LDI extracts corresponding to 10 µg protein were mixed with LD and assayed for remaining LD activity. Each value represents the mean ± SE of three replicate experiments.

1-6: six individual grains; wt: wildtype; MW: molecular weight marker sizes in kD are 97.4, 66.2, 45, 31, 21.5 and 14.4; λ: λ/Hind III molecular weight marker; -ve: negative control of PCR.

Figure 5



(A) 12% SDS-PAGE of LDI extracts corresponding to 5 μ g protein.
(B) Immunoblot developed with antiserum against LDI; bar represents 21.5 kD.

U3: homozygous T_2 generation transgenic line
U3; U4: homozygous T_2 generation transgenic line
U4; wt: wildtype; MW: molecular weight marker sizes in kD are 97.4, 66.2, 45, 31, 21.5 and 14.4.

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Figure 6

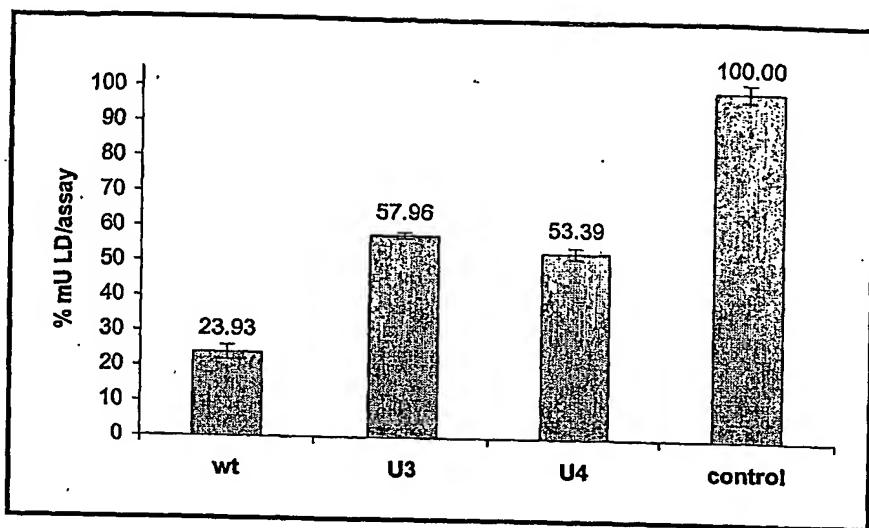
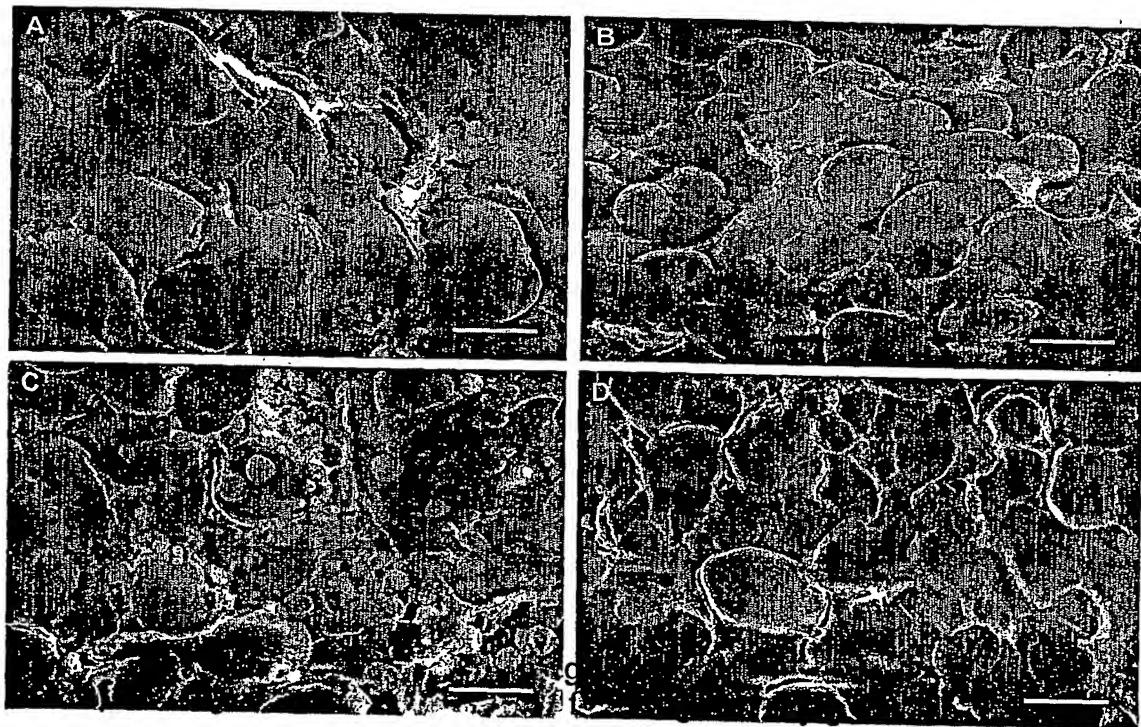


Figure 7

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(A) wildtype barley grain.

(B) transgenic T₂ generation grain of homozygous line U4.(C) wildtype T₁ grain of heterozygous transgenic line U3.(D) transgenic T₁ grain of heterozygous line U3.Bar is 10 μ m long.

Figure 8

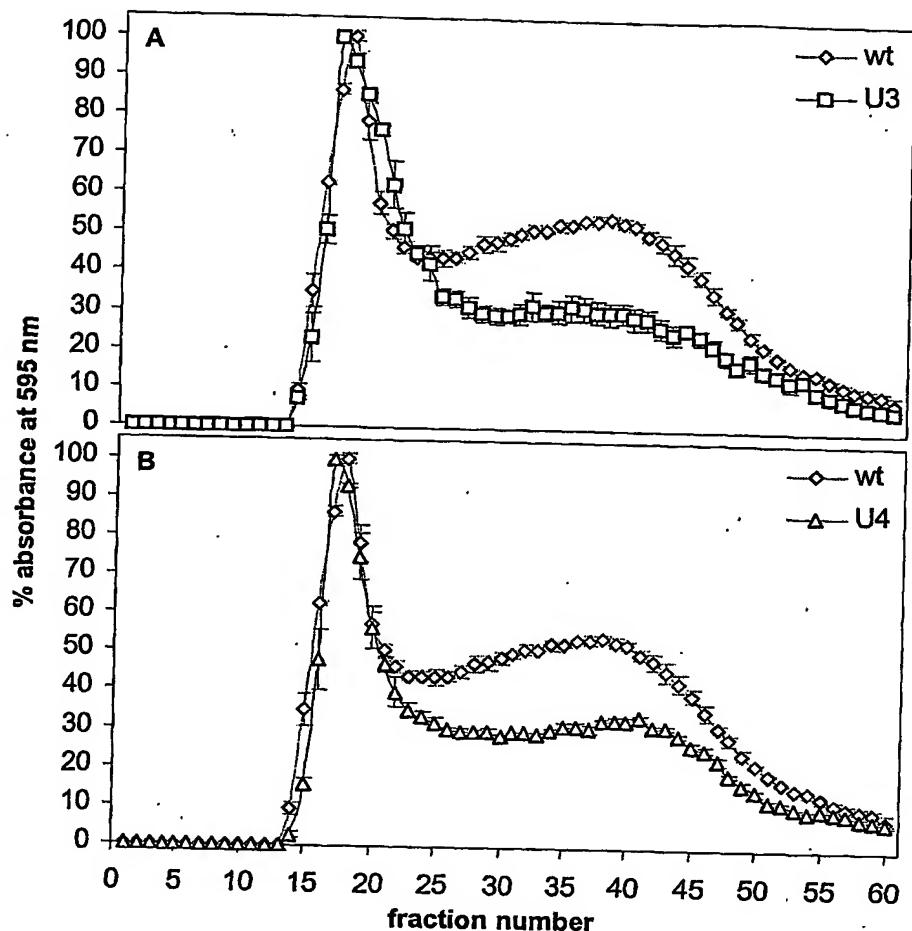


Figure 9

DNA % Identity	<i>Hordeum vulgare</i> SEQ. ID. No. 3	<i>Hordeum vulgare</i> SEQ. ID. No. 1	<i>Triticum aestivum</i>	<i>Zea mays</i>	<i>Oryza sativa</i>	<i>Hordeum spontaneum</i>
<i>Hordeum vulgare</i> SEQ. ID. No. 3	100	76.34				
<i>Hordeum vulgare</i> SEQ. ID. No. 1			77.27	63.45	42.35	43.09
<i>Triticum aestivum</i>	98.84	100				
<i>Zea mays</i>			100	65.38	45.57	46.05
<i>Oryza sativa</i>				100	41.37	46.81
<i>Hordeum spontaneum</i>					100	49.28
						100

% amino acid identities	<i>Hordeum vulgare</i> SEQ. ID. No. 4	<i>Hordeum vulgare</i> SEQ. ID. No. 2	<i>Hordeum spontaneum</i>	<i>Oryza sativa</i>	<i>Triticum aestivum</i>	<i>Zea mays</i>	<i>Secale cereale</i>	<i>Eleusine coracana</i>
<i>Hordeum vulgare</i> SEQ. ID. No. 4	100							
<i>Hordeum vulgare</i> SEQ. ID. No. 2	98.64	100	42.86	34.01	80.95	53.06	40.14	42.18
<i>Hordeum spontaneum</i>	43.54	100	29.92	43.54	48.98	66.67	46.26	
<i>Oryza sativa</i>	33.75	25.63	100	31.88	33.13	23.75	25	
<i>Triticum aestivum</i>	78.43	41.83	33.33	100	54.25	39.22	39.22	
<i>Zea mays</i>	50.97	46.45	35.48	53.55	100	43.87	52.9	
<i>Secale cereale</i>	48.36	79.51	29.51	50.82	55.74	100	62.3	
<i>Eleusine coracana</i>	51.22	54.47	29.27	50.41	66.67	61.79	100	